

GENETIC DETERMINANTS OF RABBIT BEHAVIOR IN THE LIGHT OF LITERATURE RESEARCH

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Abstract

The aim of this paper was to review the available literature for reports concerning the hereditary determinants of rabbit behavior. The available literature indicates that rabbit behavior is strongly associated with environmental conditions, but also with genetic determinants. Under natural conditions these animals display different behavioral patterns than under farm conditions; moreover, animals with a less wild behavioral pattern are characterized by better performance traits. In behavioral tests, farm rabbits exhibit behaviors such as curiosity, aggression and fear. It has been scientifically demonstrated that the heritability of cortisol concentration in rabbit blood plasma before and during exposure to stress is very low, which makes selection in this direction considerably more difficult. In addition, the value of the genetic correlation indicates that different genes may be responsible for blood cortisol concentration. In studies in which rabbits were selected for high and low activity in the open field, repeated and increasingly pronounced fear-related behavior was observed in animals from the low-activity line; moreover, molecular studies identified a minisatellite allele specific to this group. On the basis of the literature analysis, it was concluded that rabbits inherit behavioral traits and resistance to stress, but the final response of the organism is determined by environmental influences. Research shows that selection of rabbits for the expression of certain behaviors may attenuate or intensify those behaviors; however, the final effect of selection cannot be predicted with certainty because various factors determine the development of a given behavior. The available literature indicates that issues related to the inheritance of rabbit behavior are still poorly understood and therefore require further study.

Keywords: behavior, rabbit, heritability

Introduction

Increasing attention is currently being paid to the welfare of animals kept under farm conditions. This results from growing public awareness of animals' needs, not only physical needs (a sufficiently large cage/pen and permanent access to water), but also psychological needs (the possibility of expressing species-specific behavior). Every animal behaves in accordance with its psychological state (Kania, 2017), and the reactions that make up animal behavior are manifestations of its interactions with the environment (Sadowski, 2016). Animals communicate their needs to the owner or breeder through their behavior. The basis of animal rights is the fact that animals experience emotions. An animal, as a living

being capable of suffering, is not a thing; humans owe it respect, protection and care, as confirmed, among others, by the Polish Animal Protection Act of 21 August 1997. In animals, psyche is defined as the individual manner in which an individual of a given species responds, differing from the responses of other individuals. Such responses are stable over time and are not necessarily strictly situation-dependent. When an animal exhibits a larger number of interrelated behavioral responses, this is defined as a behavioral syndrome (Kaleta, 2014).

Because animals differ in personality, each individual responds differently to a given stimulus. Depending on the type and intensity of the stimulus, an animal may react with shyness, a tendency to explore, fear or aggression. Every novel stimulus triggers a stress response, which is necessary and useful because it motivates the animal to take appropriate action and may even save its life. Prolonged stress, however, leads to exhaustion and deterioration of the organism. In animal production, stress is undesirable and reduces production performance. Animals that are hypersensitive to potentially stressful factors or unable to adapt to the farming environment (which itself also causes stress) are therefore undesirable.

Rabbits are often regarded as timid animals in response to many stimuli. This is associated with their natural adaptation for escape and lack of adaptations for fighting predators. On commercial farms, stressors negatively affect reproductive performance, behavior and physiology. Timid males have low blood testosterone concentrations, which adversely affects mating and its results. Calmer females are characterized by better fertility and maternal parameters (Kowalska and Piechocka-Warzecha, 2015). Animals that are excessively timid or respond aggressively under stressful conditions should be culled from the herd. There are currently more than 100 rabbit breeds worldwide, kept for meat and fur, as companion animals and as laboratory animals. Rabbits are widely used in biomedical research and have proved to be invaluable models in immunological studies, including antibody production, immunogenetics and research on various human diseases. The Broad Institute completed the rabbit genome and transcriptome project (RabGTD; 7× coverage), identifying and assigning genomic variants from 79 rabbits (33 wild and 46 domestic). This project is expected to support not only immunological research but also studies of many diseases, such as hyperlipidemia and atherosclerosis (Carneiro et al., 2014).

On the basis of current knowledge, it was hypothesized that rabbit behavior and resistance to stressors may be inherited. The aim of this paper is to review the available Polish and international literature concerning reports on hereditary determinants of rabbit behavior.

Behavior of wild and farmed rabbits

Animal behavior depends to a large extent on environmental conditions. Behavior provides basic information about an animal's health status. Poor physical or psychological condition has a significant effect on production performance; therefore, analyzing behavior under different environmental conditions is important because these conditions produce different behavioral patterns.

Wild rabbits are approximately the size of domestic dwarf rabbits. Their body length ranges from 38 to 48 cm and body weight from 1.5 to 2.5 kg. The coat is grey-brown, providing excellent camouflage, and is lighter on the ventral and tail side. The eyes are usually dark and positioned on both sides of the head. The ears are long (up to 8 cm) and can be moved independently, allowing rabbits to listen in several directions at once; well-muscled hind limbs enable rapid escape. Wild rabbits live in groups. A colony has a hierarchy dominated by a rabbit pair: the alpha male dominates the other males and the alpha female the other females. Higher-ranking animals have priority in choosing safer burrow sites, whereas lower-ranking individuals must use areas more exposed to predators. Fights over social position occur frequently, particularly during the breeding season, and concern rank, access to females and safer burrows.

The territory occupied by a rabbit colony may cover as much as one square kilometer. Colonies most often occur at forest edges, field margins and clearings because of diverse vegetation and suitable soil for digging burrows. Wild rabbits can also be found in urban parks, cemeteries, gardens and even near housing estates. Rabbits recognize one another by pheromonal scent from glands beneath the chin. These secretions provide information about sex, age, the presence of young and even colony membership. By rubbing, rabbits also mark important objects such as burrow entrances, objects inside the burrow and other rabbits. Rabbit burrows have complex architecture, with long tunnels and several entrances and emergency exits concealed by vegetation. Inside there are sleeping areas lined with dry grass.

Rabbits regularly defecate and urinate throughout the day at designated places on the edge of their territory. Coprophagy occurs only at night and in the early morning and takes the form of cecotrophy, a two-stage method of digestion. Cecotrophy consists in lagomorphs consuming soft night feces originating from the cecum so that they can be digested again in the stomach. This process allows the animal to absorb protein nitrogen and B-group vitamins that were not digested during the first passage through the digestive tract. Wild rabbits spend most of the day resting in underground burrows. They emerge to forage after dusk and in the morning, feeding on small herbaceous plants, bark, and shoots of trees and shrubs. In winter they consume dry grass, tubers, roots, bark and shoots. They prefer intensively used pastures and forest edges.

Rabbits warn one another of danger by thumping the ground; the sound can also be heard in underground burrows, so the whole colony is alerted to an approaching predator. The breeding season of wild European rabbits lasts from February to August. Gestation lasts about 30 days and the first litters are born in early March. Rabbits form stable pairs. A pair lives and sleeps together in one burrow, forages together and rears the young. One pair produces about five litters per year. Before parturition, the female digs a burrow away from other animals and builds a nest from grass, leaves and fur plucked from her abdomen and legs. She gives birth to about five young, cleans them, consumes the placenta and umbilical cord and then nurses them. The female nurses only once or twice a day, for 2–5 minutes each time, during which the kits may consume milk amounting to 20% of their body weight (Varga Smith, 2013). After nursing, the female leaves the nest and covers it with grass, soil and leaves.

Rabbit kits are altricial: they are born blind and deaf and without a hair coat, although they have a well-developed sense of smell. Their first fur begins to grow after three days. After one week they begin to hear, and after ten days they can see. At about 24 days the female leaves the young under the care of the male. Rabbits reach sexual maturity in the fourth month of life. Males are then driven out of the colony and leave to join another colony or establish their own. Wild rabbits may live up to 10 years, although this is uncommon because of disease and predation (McBride, 2003).

The environment in which rabbits are kept for breeding differs markedly from their natural habitat, and the behavior of domestic rabbits has therefore changed relative to that of wild rabbits. Gacek (2000) identified similarities and differences between wild and farmed rabbits and distinguished a number of important, typical responses arising from rabbit biology and closely related to the environment. Farmed rabbits consume feed for 2.5–3 hours per day at irregular intervals. They prefer sweet-tasting feed, while females additionally prefer salty feed. Under farm conditions, animals eat simultaneously, and physically weaker individuals usually occupy less favorable positions at the feeder. Farm rabbits usually defecate and urinate regularly in a corner of the cage, and coprophagy occurs throughout the day.

Sexual behavior is comparable in wild and farmed rabbits. Before mating, animals chase, sniff and lick one another. Females rearing young display defensive and aggressive behavior when the nest is approached. Domestic does may build untidy nests, allowing kits to fall out, and after kindling they rarely lick and clean the young. Researchers have observed that nest-building ability improves up to the third parturition (Benedek et al., 2020). Domestic rabbits also show less interest in and care for their young than wild rabbits. Young rabbits imitate their mother's behavior and show a strong tendency to explore the environment. They remain with their mothers until five weeks of age and are then weaned. Males and females are kept separately. Rabbits are usually first mated at five months of age.

Both farmed and wild rabbits spend about 70% of the day resting, most often lying on the abdomen or sitting with the back held horizontally. Aggression in farm conditions depends on age and may occur in

response to unfamiliar people or frequent changes in animal-care staff. It is therefore directed primarily toward humans; cases of rabbits biting one another are rare. Fear behavior mainly consists of panic running around the cage as an attempt to escape, whereas thumping the floor to warn others of danger is very rare.

Genetic determinants of rabbit behavior

Behavioral tests can be used to distinguish groups of animals with different personalities. This is extremely useful when selection is carried out for a particular personality trait. In studies on the heritability of behavioral patterns, such tests are a practical, helpful and frequently used experimental tool.

Blood plasma cortisol concentration may be regarded as an indicator of stress level (Maj et al., 2001; Traczyk and Trzebiski, 2012; Badowska-Kozakiewicz, 2013). Maj et al. (2001) therefore attempted to estimate cortisol and corticosterone concentrations in rabbits from three generations designated P (parental generation), F1 (first filial generation) and F2 (second filial generation). The stressor consisted of suspending the animals for 15 minutes in a plastic net 1.2 m above the ground. Blood was collected from the marginal ear vein into heparinized tubes before exposure to the stressor and during the final, 15th minute of the test.

The authors observed no significant differences in cortisol concentration among generations before the stressor: P – 49.06 nmol/L, F1 – 46.05 nmol/L and F2 – 51.66 nmol/L. However, in the 15th minute of the test a significant difference was found between F1 (100.26 nmol/L) and F2 (81.96 nmol/L). Rabbit age did not significantly affect cortisol concentration, whereas sex had a major effect. Both before the test and in its 15th minute, females had much higher blood cortisol concentrations, indicating lower resistance to stress than males. The authors estimated heritability (h^2) of cortisol concentration at 0.20 before stress and 0.08 in the 15th minute of stress. The genetic correlation between cortisol level before exposure to the stressor and in the final minute of the test was estimated at $r_G = 0.92$. On the basis of the genetic correlation between cortisol concentrations before and during exposure to the stressor, the authors concluded that different genes may be responsible for these two traits. They also concluded that “the range of genetic variation in pre-stress cortisol concentration is sufficiently large to permit effective selection aimed at reducing the value of this trait.”

Daniewski and Jezierski (2003) conducted divergent selection of two New Zealand rabbit lines for high and low activity in the open field. Of 60 males and 60 females tested, 20 males and 20 females with the highest scores (the greatest number of squares crossed with both forepaws) were selected as founders of the first line, while 20 females and 20 males with the lowest scores (zero) founded the second line. Because most low-scoring animals obtained a score of zero, an additional selection criterion was the number of movements in the starting square (movement of all four paws). Selection was continued for eight generations. Each generation was tested in the open field, recording the number of movements in the starting square, latency in seconds to leave the starting square, the open-field score corresponding to the number of squares crossed with both forepaws, the number of movements in the starting square per minute, and the number of squares crossed with both forepaws per minute.

No major sex differences were found except in the number of movements in the starting square and movements per minute; females also showed slightly greater mobility. In the low open-field line, animals reached stable results after the sixth generation: generation six and all subsequent generations scored zero in the open-field test. In the high open-field line, mean locomotor activity increased up to generation three, fell markedly in generation four, increased in generation seven and in generation eight again declined to the level obtained by the first generation after selection. In both lines, the number of movements in the starting square decreased significantly in successive trials, with a stronger trend in the high-scoring line. The number of movements per minute increased in the high-scoring line, whereas the number of squares crossed per minute did not change substantially in successive tests.

The authors estimated heritability (h^2) of open-field scores in the low-scoring line for generations 0–3 and 0–8 as 0.46 and 0.44, respectively. In the high-scoring line, heritability for generations 0–3 was 0.23; it could not be calculated for generations 0–8 because data variability was too high for reliable estimation. Rabbits with low open-field scores had higher body weights than rabbits with high scores. The largest between-line differences in body weight occurred before testing, at 4–8 weeks of age, and between 14 and 16 weeks. No significant sex differences in body weight were found except at eight weeks, when males were considerably heavier. The researchers noted that the greatest body-weight gain in the fourth generation coincided with a decrease in locomotor activity in the high-activity line. Within that line, body-weight gain also tended to decrease in successive generations, as shown by a regression coefficient of 0.094.

Marked differences between the high- and low-scoring lines were also found in the percentage of females producing offspring relative to the number of females mated. A higher proportion of pregnant females occurred in the low-scoring line. In the high-scoring line, pronounced decreases in successful pregnancies were observed in generations three and six. No significant between-line differences were found in the numbers of live- and stillborn young before the end of weaning, although differences occurred among successive generations within both lines. The authors concluded that selection for high and low open-field activity was in fact selection for active and passive coping styles. In nonsocial situations, actively coping animals respond more rapidly to unfamiliar objects than passively coping animals. Passively coping animals respond to both social and nonsocial situations with withdrawal and immobility, and this response is usually repeated consistently in similar situations. Cage housing reduces locomotor activity and environmental variability and is therefore more disadvantageous for actively coping animals. Animals showing active coping, i.e. higher open-field activity, have greater difficulty adapting and expressing behavior under cage conditions, resulting in lower body weight, a lower fertilization rate and poorer maternal parameters.

Molecular genetic methods make it possible to identify specific genes responsible for traits. Examples of genes affecting personality include DRD4, located on human chromosome 11, and 5-HTT, located on human chromosome 17 (Kaleta, 2014). DRD4 encodes the D4 subtype of the dopamine receptor. It contains numerous polymorphic sites composed of tandem repeats. Mutations within this gene have been associated with various behavioral phenotypes as well as autonomic nervous system dysfunction, attention disorders and hyperactivity (U.S. National Library of Medicine – NCBI DRD4 Gene). The 5-HTT gene encodes the serotonin transporter. Mutations associated with this gene may alter transporter function. A promoter variant of the serotonin transporter gene, consisting of long and short variants, is considered one of the factors contributing to the onset of many psychiatric disorders.

According to the literature, a variable-length repeat in the promoter region may affect the rate of serotonin uptake and may consequently play a role in post-traumatic stress disorder, susceptibility to depression, anxiety disorders and even suicidal behavior (Kuzelova et al., 2010). Studies using laboratory animals are currently being conducted on the effects of genetic factors on personality traits, with rodents and primates being of particular interest. Specially selected animal lines differing in behavioral traits are used as research models in pharmacological studies and in therapies for psychiatric disorders (Kaleta, 2014). To date, the genes mentioned above have not been analyzed in rabbits.

DNA fingerprinting makes it possible to identify an individual from a DNA sample by analyzing unique DNA patterns using minisatellites. Minisatellites are genomic regions containing repeated nucleotide sequences 6 to about 100 base pairs long. These regions show a high degree of population variability; consequently, fingerprinting based on the detection of minisatellites produces a unique individual pattern – a genetic fingerprint. Sacharczuk et al. (2005) selected rabbits for high and low open-field activity over eight generations. The open field was chosen as the basis of selection because, as the authors noted after Crawley et al. (1997), no single trait reflects emotional activity or anxiety behavior; rather, genes underlying these traits are simultaneously involved in locomotor activity, exploratory behavior, anxiety behavior and other emotional manifestations in the open-field test.

For DNA fingerprinting analysis, 14 males were randomly selected from the high-activity line and the same number from the low-activity line. In each group, equal amounts of DNA from ten rabbits were pooled to create a common pattern representative of the line. The remaining four DNA samples were used to create individual genetic patterns defining the range of band separation and other genetic parameters within each line. The authors found no significant difference in genetic variability parameters. Analysis of bands from individual animals and pooled representative samples identified a specific minisatellite allele in the low open-field activity line. No such alleles were found in the high-activity line. In addition, heritability of active behavior in the high-activity line decreased during selection. Mutations within minisatellites may affect protein expression and even produce pathological protein forms. The researchers drew attention to minisatellites within serotonin- and dopamine-transporter or receptor genes, where such mutations could significantly increase susceptibility to depression and social or neurotic disorders.

Summary

Rabbit behavior is strongly associated with environmental conditions as well as inherited genes. Under natural conditions rabbits display behavioral patterns different from those observed under farm conditions, and animals with less wild behavior are characterized by better performance traits. Traits necessary for survival in nature may become problematic in farming. In the natural environment, exploratory behavior is essential for survival, whereas studies have shown that under farm conditions rabbits with a greater need to explore, because of insufficient stimulation, lose body weight, are less fertile and prolific, and show poorer maternal traits.

Females with a weak maternal instinct build untidy nests or no nests at all and are reluctant to care for their offspring, resulting in kit mortality. Conversely, aggressive mothers are a significant management problem because they make routine husbandry procedures difficult, such as checking litter condition and nest quality or replacing bedding. Under natural conditions stress is a motivating factor, whereas under farm conditions it is undesirable and may lead to disease or even death. Changes in living conditions have led rabbits to develop different behavioral traits that facilitate adaptation to farming and meet breeders' requirements; aggressive, dangerous or inefficient animals are often eliminated and therefore do not pass on their genes.

Studies have shown that rabbits can be selected for a given behavioral pattern. In rabbits with low open-field activity, the passive-defensive behavioral pattern may become increasingly pronounced, with reluctance and fear of exploring new areas increasing from generation to generation. In rabbits with high open-field activity, by contrast, the heritability of this behavioral pattern decreased, as did activity and willingness to explore the field. Selection for specific behaviors may attenuate or intensify them, but the final outcome is uncertain because many factors determine the development of behavior. Rabbits inherit temperament from their ancestors, but it is ultimately shaped by the environment in which the animal lives. The available literature indicates that the inheritance of rabbit behavior is poorly understood and that further research is therefore needed.

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